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Product no AS09 458

## Anti-PEPC | Phosphoenolpyruvate carboxylase

## **Product information**

Immunogen

KLH-conjugated synthetic peptide well conserved PEPC1 and sequences from different plant species including Arabidopsis thaliana Q9MAH0, At1g53310 (PEPC 1), Q84VW9, At3g14940 (PEPC 3). The peptide chosen to elicit this antibody is also perfectly conserved in bacterial type of this enzyme NP 177043.2 (PEPC 4).

For Zea mays, the peptide is converved in PEP1 and PEP4 isoforms.

**Host** Rabbit

Clonality Polyclonal

**Purity** Immunogen affinity purified serum in PBS pH 7.4.

Format Lyophilized

Quantity 50 μg

**Reconstitution** For reconstitution add 50 μl of sterile water

Storage

Store lyophilized/reconstituted at -20 °C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube. Please do not re-use this primary antibody solution. In case of cyanobacterial samples there will be no signal in your second incubation.

Additional information

PEPC is especially prone to proteolysis. To avoid artifacts, we use chymotrypsin, as recommended by Plaxton (2019), in addition to the Roche protease inhibitor cocktail.

## **Application information**

**Recommended dilution** 1:500 (IL), 1:1000 -: 10 000 (WB)

Expected | apparent

Confirmed reactivity

110 | 105 kDa

Ananas comosus, Arabidopsis thaliana, Cenchrus ciliaris, Chenopodium quinoa, Chloris gayana, Chromera velia, Cyanthobasis fruticulosa, Glycine max, Hordeum vulgare, Jatropha curcas, Kochia prostrata, Leptochloa fusca, Lupinus sp., Megathyrsus maximus, Mesembryanthemum crystallinum, Nicotiana tabacum, Oryza sativa, Panicum antidotale, Panicum coloratum, Petrosimonia nigdeensis, Pinus strobus, Saccharum spp. hybrid clone C91-301, Salsola lanata, Salsola laricifolia, Salsola grandis, Salsola tragus Sorghum bicolor, Synechocystis PCC 6803, Phaeodactylum tricornutum (strain CCAP 1055/1), Pinus strobus, Thalassiosira weissfloggi, Zea mays, Zostera muelleri

Predicted reactivity

Brassica napus, Cucumis sativus (PEPC1, PEPC2, PEPC3), Flaveria bidentis, Flaveria trinervia, Lupinus albus, Mammillaria thornberi, Manihot esculenta, Manihot obovata, Medicago sativa, Morinda citrifolia, Nannochloropsis gaditana CCMP526, Nopalea gaumeri, Opuntia macbridei, Pachycereus pringlei, Saccharum spp, Solanum tuberosum, Spinacia oleracea, Streptanthus tortuosus, Pachycereus hollianus, Pisum sativa, Phaseolus vulgaris, Populus sp., Triticum aestivum, algae, diatoms: Thalassiosira pseudonana, other species: Salmonella sp., Schiedea hookeri, Shigella sp. Schiedea sarmentosa, Solanum lycopersicum, Streptanthus farnsworthianus, Tacinga saxatilis, Yersinia sp. Vibrio sp., Quercus sp.

Species of your interest not listed? Contact us

Not reactive in Methanothermobacter thermautotrophicus

Additional information Antibody can be also used following 2D gel electrophoresis

Selected references

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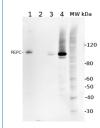
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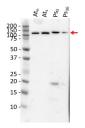
Elevated Concentration of ??2. Russ J Plant Physiol 68, 1107–1114 (2021). https://doi.org/10.1134/S1021443721060169

<u>Durall</u> et al. (2020). Increased ethylene production by overexpressing phosphoenolpyruvate carboxylase in the cyanobacterium Synechocystis PCC 6803. Biotechnol Biofuels. 2020 Jan 28;13:16. doi: 10.1186/s13068-020-1653-y.



5 μg of total protein from (1) *Arabidopsis thaliana* leaf extracted with Protein Extration Buffer, PEB (AS08 300), (2) *Spinacia oleracea* total cell, extracted with PEB, (3) *Hordeum vulgare* total cell extracted with PEB, (4) Zea mays total cell extracted with PEB, were separated on 4-12% NuPage (Invitrogen) LDS-PAGE and blotted 1h to PVDF. Blots were blocked immediately following transfer in 2% blocking reagent (GE Healthcare) in 20 mM Tris, 137 mM sodium chloride pH 7.6 with 0.1% (v/v) Tween-20 (TBS-T) for 1h at room temperature with agitation. Blots were incubated in the primary antibody at a dilution of 1: 10 000 for 1h at room temperature with agitation. The antibody solution was decanted and the blot was rinsed briefly twice, then washed once for 15 min and 3 times for 5 min in TBS-T at room temperature with agitation. Blots were incubated in secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated) diluted to 1:50 000 in 2% blocking solution for 1h at room temperature with agitation. The blots were washed as above and developed for 5 min with chemiluminescent detection reagent according the manufacturers instructions. Images of the blots were obtained using a CCD imager (FluorSMax, Bio-Rad) and Quantity One software (Bio-Rad).

**PEPC** is especially prone to proteolysis. To avoid artifacts, we use chymotrypsin, as recommended by <u>Plaxton (2019)</u>, in addition to the Roche protease inhibitor cocktail.



10  $\mu$ g of total protein extracted freshly from *Arabidopsis thaliana* wt leaf tissue (At<sub>n</sub> non-senescent leaves), *Arabidopsis thaliana* wt leaf tissue (At<sub>s</sub> senescent leaves), *Pinus strobus* needle tissue (PS<sub>0</sub> PS<sub>36</sub>) with 1 M Tris-HCl, pH 6.8, 10 % SDS, 15 % sucrose, 0.5 DTT and denatured at 75 °C for 5 min. were separated on 10 % Bis-Tris Nupage Novex gel (120 V/45 min. using MES buffer system) and blotted 30 min. to PVDF. Blot was blocked with 5 % non-fat milk 45 min./RT with agitation. Blot was incubated in the primary antibody at a dilution of 1: 1000 for 1h/RT with agitation in TBS with 2 % non-fat milk or ON/4 °C with agitation. The antibody solution was decanted and the blot was rinsed briefly twice for 10 min. in TBS at RT with agitation. Blot was incubated in Agrisera matching secondary antibody (anti-rabbit IgG horse radish peroxidase conjugated, <u>AS09 602</u>) diluted to 1:75 000 in for 1h/RT with agitation. The blot was washed as above and developed using chemiluminescent detection. Exposure time was 40 seconds.

Courtesy of Dr. Christine Yao-Yun Chang and the Ensminger lab, University of Toronto, Canada